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Human Insulinoma: Clinical, Cellular, and Molecular Aspects*

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Abstract

Insulinoma is the most frequently encountered functioning endocrine pancreatic tumor in humans. In this overview we summarize morphological and clinical features of insulinomas, report about the proinsulin–insulin conversion in normal and neoplastic B-cells, discuss the new classification, the criteria of malignancy, and the clonal composition of endocrine pancreatic tumors, and outline recent findings on the molecular pathology of these tumors.

Key Words: Pancreas, insulin, molecular pathogenesis

Introduction

Endocrine pancreatic tumors (EPTs) have a prevalence of approx 1 in a population of 100,000 and an incidence of 1 per 10⁶ per year for insulinomas and gastrinomas, and one of 1 per 10⁷ per year for the other tumors.

EPTs giving rise to well-defined hormonal syndromes are designated “functioning” and are classified according to the hormone responsible for the clinical syndrome. “Nonfunctioning” (nonsecreting, inactive, hormonally silent) EPTs do not lead to endocrinological symptoms and may be named according to the major product detected by immunohistochemistry or radioimmunoassay, i.e., “EPT with production of insulin.”

In surgical series, functioning EPTs account for 60–85% of all EPTs [1]. Clinically unrecognized and asymptomatic tumors, usually smaller than 1 cm in diameter, are found in 0.4–1.5% of un-

lected autopsies [2]. In surgical series, nonfunctioning tumors comprise 15–21% of all EPTs [1]; in our series of 501 tumors, it is the largest group representing 34% of all EPTs [3]. Approximately 25% of all EPTs are associated with multiple endocrine neoplasia type 1 (MEN1).

Although previously thought to arise from neural ectoderm, the neuroendocrine cells of the gastrointestinal system, including the pancreas, are derived from endoderm [4,5]. However, whether EPTs originate from the ductular epithelium or the islet cells of the pancreas is still a matter of debate [6].

Precise classification of EPTs requires analysis of the cell phenotype by immunohistochemistry (Tables 1 and 2). Because the tumors originate from cells that are “phenotypically” neuroendocrine, they share broad spectrum markers for neuroendocrine differentiation, such as neuron-specific-enolase (NSE), synaptophysin, and

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chromogranin A. In the majority of functioning tumors, the hormone causing the syndrome can be detected by immunohistochemistry at the light and electron microscopic level. Staining intensity or number of positive cells, however, are not related to the severity of symptoms. Using a panel of antibodies against pancreatic hormones, many tumors turn out to be multihormonal. Nonfunctioning tumors frequently exhibit positivity for a variety of hormones, without causing clinical symptoms.

Clinical Features of Insulinomas

Symptoms of hypoglycemia due to hypersecretion of insulin were first described by Harris in 1924 [7], and three years later the association between insulin-secreting EPTs and hypoglycemia was recognized by Wilder et al. [8].

Insulinomas are by far the most frequent of all functioning EPTs (prevalence: 1–1.25 per 10⁶ of people; incidence: four patients per 10⁶ person years) [9,10]. Insulinomas have been diagnosed in all age groups, but rarely occur in adolescence. The highest incidence is found between 40 and 60 yr, with an average age of 44–46 yr. Approximately 10% of the patients are younger than age 20 and 10% older than 60. There is 60:40 ratio of females to males in most reported series [1].

It is generally accepted that the vast majority of insulinomas are benign. The percentages of malignant tumors range from 2.4 to 17.9%, with an average of 8.4% [10–14]. Malignant insulinomas occur in an older age group and have not been reported in children [15]. It appears that males are more frequently affected than females [16].

Virtually all functioning insulinomas manifest with symptoms of hypoglycemia, which include adrenergic and hypo-gly-

copenic signs [17]. A gradual onset of sweating and palpitations (warning symptoms) in hypoglycemia is typical. More than 80% of patients have transient symptoms of CNS dysfunction, ranging from drowsiness to coma and epilepsy, and there are several reports of insulinoma patients with erroneous psychiatric or neurologic diagnoses [13].

The diagnosis of an insulinoma is mainly based on laboratory examinations and provocative tests. Inappropriate insulin values in relationship to the plasma glucose level are key characteristics of hyperinsulinemic hypoglycemia due to an insulinoma. In healthy individuals the plasma insulin is suppressed to less than 6 μ U/mL during hypoglycemia (≤ 40 mg/dL; 2.2 mM) and the ratio of glucose to insulin is greater than 2.5. The measurement of plasma proinsulin can also be helpful in the diagnosis of insulinoma. The majority of patients present proinsulin levels of more than 22% of the total plasma insulin, and it has been shown that especially malignant tumors present high plasma proinsulin levels [18,19].

Provocative tests include monitored fasting and secretagogue stimulation of insulin by tolbutamide, glucagon, or calcium. The demonstration of low plasma glucose, and elevated or normal insulin in the absence of ketonuria suggests an insulinoma. Insulinoma patients typically demonstrate unchanged C-peptide levels, owing to continuous insulin secretion by the tumor [20].

Methods of localizing insulinomas include noninvasive techniques such as ultrasonography, bolus-enhanced computed tomography (CT), magnetic resonance imaging, and octreotide scan, as well as invasive techniques such as arteriography with or without subtraction and transhepatic portal venous sampling.

Table 1. Classification of EPT

1. Well-differentiated endocrine tumor
1.1. Functioning
Insulin-producing (insulinoma)
Glucagon-producing (glucagonoma)
Somatostatin-producing (somatostatinoma)
Gastrin-producing (gastrinoma)
VIP-producing (vipoma)
Serotonin producing tumor with carcinoid syndrome
Others
1.2. Nonfunctioning
Microadenoma (<0,5 cm)
Others
2. Well-differentiated endocrine carcinoma
2.1. Functioning (see 1.1. for types)
2.2. Nonfunctioning
3. Poorly differentiated endocrine carcinoma
4. Mixed exocrine–endocrine tumor
5. Tumor-like lesions
Islet hyperplasia
Nesidioblastosis
Endocrine dysplasia

Table 2. Clinicopathologic Correlations

1. Well-differentiated endocrine tumor
benign behavior: confined to the pancreas, nonangioinvasive, < 2 cm in size [*]
Functioning
Insulinoma
Nonfunctioning
uncertain behavior, confined to the pancreas; ≥ 2 cm in size or angioinvasive
Functioning
Gastrinoma, insulinoma, vipoma, glucagonoma, somatostatinoma or inappropriate syndrome ^{**} tumor
Nonfunctioning
2. Well-differentiated endocrine carcinoma
low grade malignant with gross local invasion and/or metastases
Functioning
Gastrinoma, insulinoma, glucagonoma, vipoma, somatostatinoma or inappropriate syndrome ^{**} tumor
Nonfunctioning
3. Poorly differentiated endocrine carcinoma
high grade malignant (small to intermediate cell) carcinoma

^{*} < 2 cm in size implies near 100% probability of benign behavior; < 3 cm corresponds to 90% probability.

^{**} Inappropriate hormone syndromes: Cushing (ACTH), acromegaly or gigantism (GRH), hypercalcemia, and so on.

Strategies for insulinoma treatment include surgical removal or debulking of tumor tissue, including liver transplantation in case of metastases [21], drug therapy aimed at palliation of symptoms referable

to hypoglycemia [22], and chemotherapy in patients with metastatic disease [23]. About two-thirds of the patients can be cured by resection of the tumor. Blind distal pancreas resection is indicated in

patients where the tumor cannot be located during operation and measurement of changes in plasma glucose during surgery is a helpful means in predicting a cure for the patient [24].

(Histo)pathological Features of Insulinomas

The majority of insulinomas occur in the pancreas or are attached to it. Extrapancratic tumors are extremely rare (1.8%) and are most commonly found in the duodenal wall [25,26]. Macroscopically insulinomas are usually red-brown in color and are softer than the surrounding pancreatic tissue, with the exception of tumors with a large amount of fibrous stroma and/or amyloid. Collective data indicate that tumors are equally distributed between the head, body, and tail of the pancreas with a slight predominance of the head and tail region [25–28]; 83% of insulinomas occur single, 11–13% multiple, and 4% are associated with MEN1 [29]. Functioning insulinomas are frequently discovered while still small and the reported mean size of the tumors has varied from microscopic up to 11 cm: 75% of the tumors are between 0.5–2 cm and weigh less than 2 g. Symptoms of hypoglycemia are unrelated to the size of tumor [18]. Only 8% of tumors are larger than 5 cm [25]. These tumors are more likely to be malignant than smaller ones.

The insulinomas producing a hypoglycemic syndrome in MEN1 patients are usually larger than 1 cm, and microadenomas with insulin expression, no matter how numerous they are, seem to remain functionally silent. This implies that the insulinomas in MEN1 patients are among the grossly apparent and palpable pancreatic tumors. If there are several large tumors, usually only one of them is an insulinoma. Multiple large insulinomas are rare.

Several histological classifications have been proposed for sporadic insulinomas [30–32]. However, the clinical relevance of such classifications is not proven. Most insulinomas store insulin and proinsulin in a sufficient amount to be easily visualized by immunohistochemistry. Typically insulin-rich cells account for 60–80% of all tumor cells. However, the amount of immunohistochemical presence of insulin does not correlate with hormone levels in the serum. About half of the insulinomas are multihormonal. In such tumors insulin-rich cells are admixed with cells producing glucagon, somatostatin, or pancreatic polypeptide. Amyloid deposition is a common finding in insulinomas and immunohistochemical detection of islet amyloid polypeptide (IAPP) or amylin, a possible causative factor for amyloid deposition, has recently been described in 16 out of 19 tumors [33].

Proinsulin–Insulin Conversion in Insulinomas

The biosynthesis of insulin commences in the endoplasmic reticulum (ER), and the preproinsulin becomes converted to proinsulin in the ER by the action of a signal peptidase. This is followed by various post-translational processing steps involving the action of two prohormone-converting endoproteases and of carboxypeptidase H. Each of the two endoproteases preferentially cleaves one of the two dibasic cleavage sites in proinsulin, followed by the removal of the C-terminal basic residue by carboxypeptidase H. Thus, two cycles of combined endoprotease–carboxypeptidase action are required to generate mature insulin and C-peptide from proinsulin. These processing steps are accompanied by the sorting and packing of insulin into secretory granules. The combination of

biochemical and immunohistochemical approaches has permitted the fine analysis of the proinsulin–insulin conversion in normal pancreatic beta cells [3]. In light microscopic studies, the proinsulin-specific monoclonal antibody GS-9 A 8, recognizing an epitope around the dibasic processing site Arg31-Arg32, resulted in a Golgi-like staining pattern (Fig. 1). By immunoelectron microscopy, this antibody provided *in situ* evidence that the proinsulin–insulin conversion takes place in partially clathrin-coated, acidic immature secretory granules. Subsequent immunoelectron microscopic studies provided evidence that the endoproteolytic cleavage at the dibasic pair Lys64-Arg65 in the A chain/C-peptide junction also occurred in the clathrin-coated immature secretory granules. These data were complemented by microscopic studies, applying a monoclonal antibody specific for insulin. By light microscopy, a diffuse cytoplasmic labeling for insulin was evident (Fig. 1). By immunoelectron microscopy, insulin immunolabeling first became detectable in clathrin-coated immature secretory granules, which upon acidification transformed in mature secretory granules and exhibited highest labeling for insulin. Thus, in normal pancreatic beta cells, proinsulin–insulin conversion occurs in acidic immature secretory granules of the trans Golgi apparatus (Fig. 2).

Insulinomas can exhibit many structural and immunohistochemical features, in common with normal beta cells. However, even within a given insulinoma, insulin immunostaining and ultrastructural details of secretory granule morphology may vary greatly. Variability is also observed in their clinical manifestations. We have investigated the distribution pattern of proinsulin and insulin by light and electron microscopic immunolabeling, applying the proinsulin and insulin-specific monoclonal

antibodies [34,35]. By light microscopy, 76 human insulinomas were studied. One trabecular and two solid insulinomas showed the staining patterns of normal beta cells. A “near normal” staining pattern, perinuclear proinsulin and diffuse or polarized insulin staining (Fig. 1), existed in 10/27 trabecular and 11/44 solid insulinomas. An “intermediate” staining pattern, intense perinuclear as well as weaker diffuse proinsulin staining with diffuse or polarized insulin staining (Fig. 1) was observed in 10/27 trabecular and 20/44 solid insulinomas. Of the five glandular insulinomas, four exhibited a “near normal” and one an “abnormal” staining pattern. Similar results were obtained by others in a recent immunohistochemical study [36], which also addressed the immunohistochemistry of the prohormone processing enzymes. In our studies, no correlation was found among any particular staining pattern, histological type, the multihormonality, or the malignancy of the insulinomas [35]. The abnormal labeling pattern for proinsulin in about 60% of the insulinomas was suggestive of topographical abnormalities of hormone conversion and an immunoelectron microscopic study was carried out in seven such insulinomas [34]. We found that, in contrast to normal beta cells, the proinsulin–insulin conversion in insulinomas occurred already in the Golgi apparatus (Fig. 3), but remained incomplete, resulting in the formation of secretory granules containing both proinsulin and insulin (Figs. 2 and 3). This indicates that in insulinoma sorting into secretory granules may not be a prerequisite for hormone conversion. It also indicates that proinsulin and insulin storage and secretion occur through secretory granules via the regulated secretory pathway. A substantial variability for both proinsulin and insulin immunolabeling in secretory granules was observed not only in individual tumor cells, but also

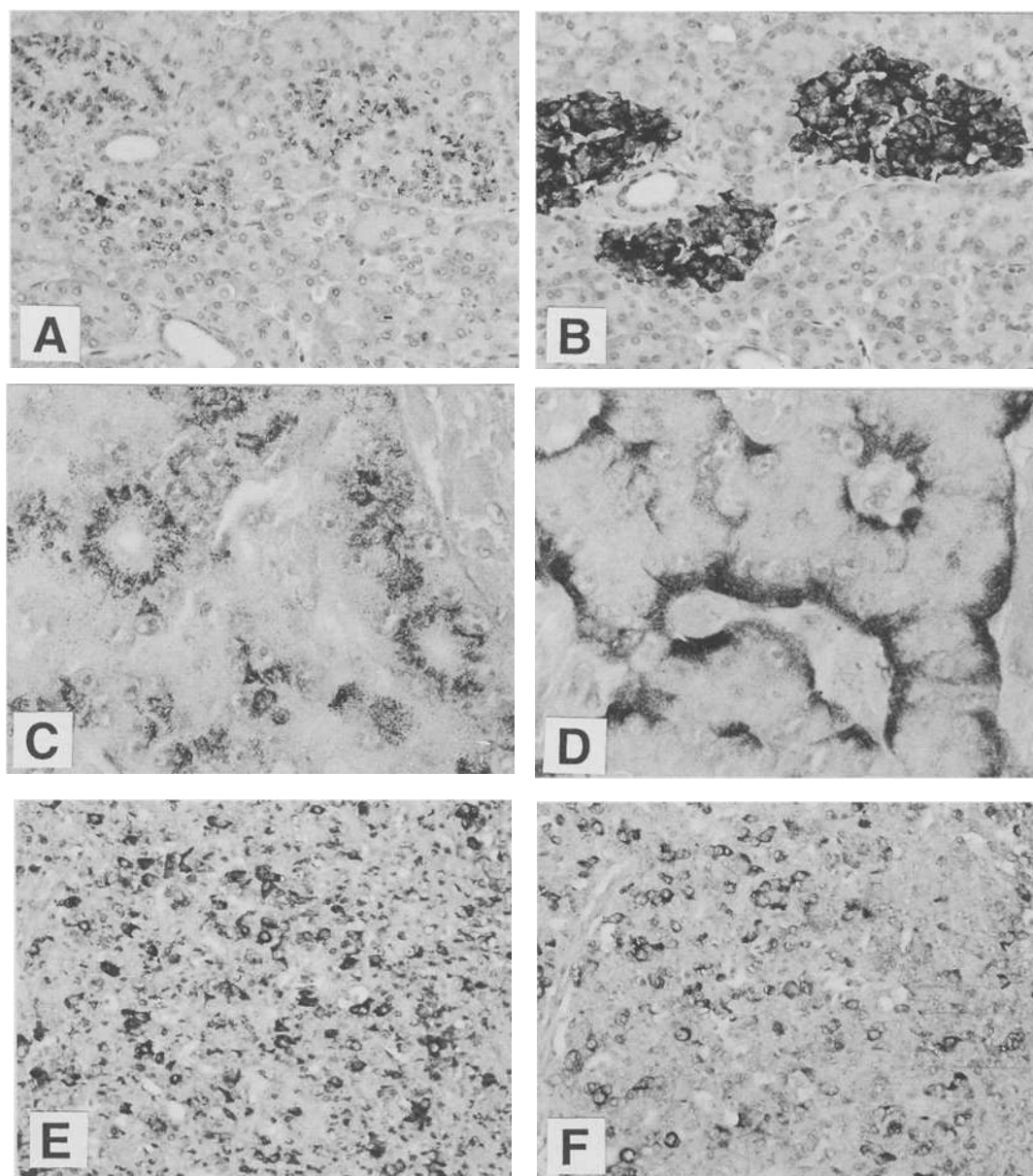


Fig. 1. Immunohistochemical detection of proinsulin (A, C, E) and insulin (B, D, F) in human pancreas and insulinomas, paraffin sections, silver-intensified immunogold staining using monoclonal antibodies specific for proinsulin and insulin. In islets of Langerhans of normal human pancreas, proinsulin immunohistochemistry results in a perinuclear crescent-shaped, Golgi staining pattern (A). Insulin immunostaining in a serial section consecutive to the one shown in (A) results in a diffuse cytoplasmic labeling (B). Insulinomas may exhibit a near normal perinuclear proinsulin staining (C) and a polarized cytoplasmic insulin staining (D). However, insulinomas may show an abnormal diffuse proinsulin staining pattern (E). Insulin immunostaining in a consecutive serial section from the insulinoma shown in (E) is presented in (F).

among the insulinomas studied. This variability may account for the lack of correlation between pathohistological, immunohistochemical, and clinical parameters in functioning insulinomas [34–36].

Criteria of Malignancy in EPTs

Based on the work of Capella et al. [37], the World Health Organization (WHO) has elaborated a new classification of neuroendocrine tumors including EPTs,

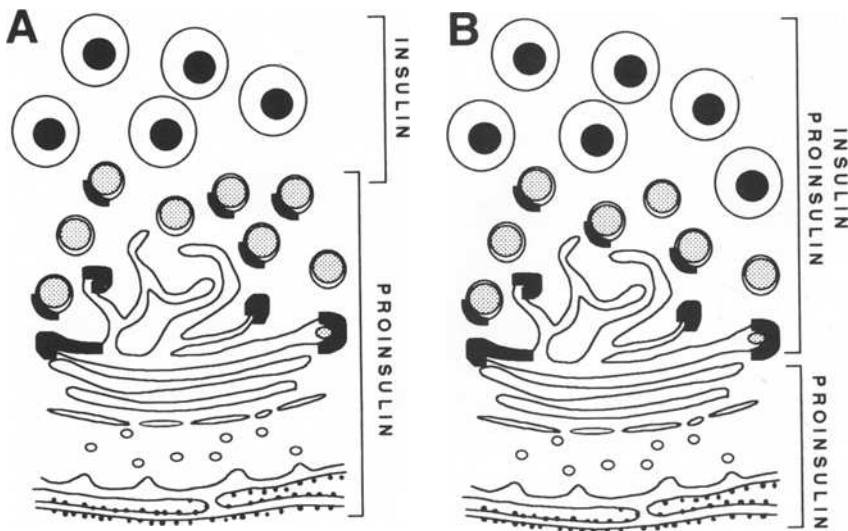


Fig. 2. Schematic presentation of proinsulin–insulin conversion in normal pancreatic beta cells (A) and human insulinoma (B). For details, see text.

which is currently in press and has been presented in review articles [38,39]. In the new classification (Table 1), tumors smaller than 2 cm in size, confined to the pancreas, without angio-invasion or metastases and with a mitotic rate of less than 2 mitoses per 10 high power fields or less than 2% Ki 67 (or MiB-1) staining index are considered benign. However, the only reliable criteria for malignancy are the infiltration of the tumor into adjacent organs (e.g., the spleen), the presence of angioinvasion of lymph node, and/or distant metastases [40].

It has been hypothesized that immuno-histochemical markers might be helpful in identifying tumors with a more aggressive

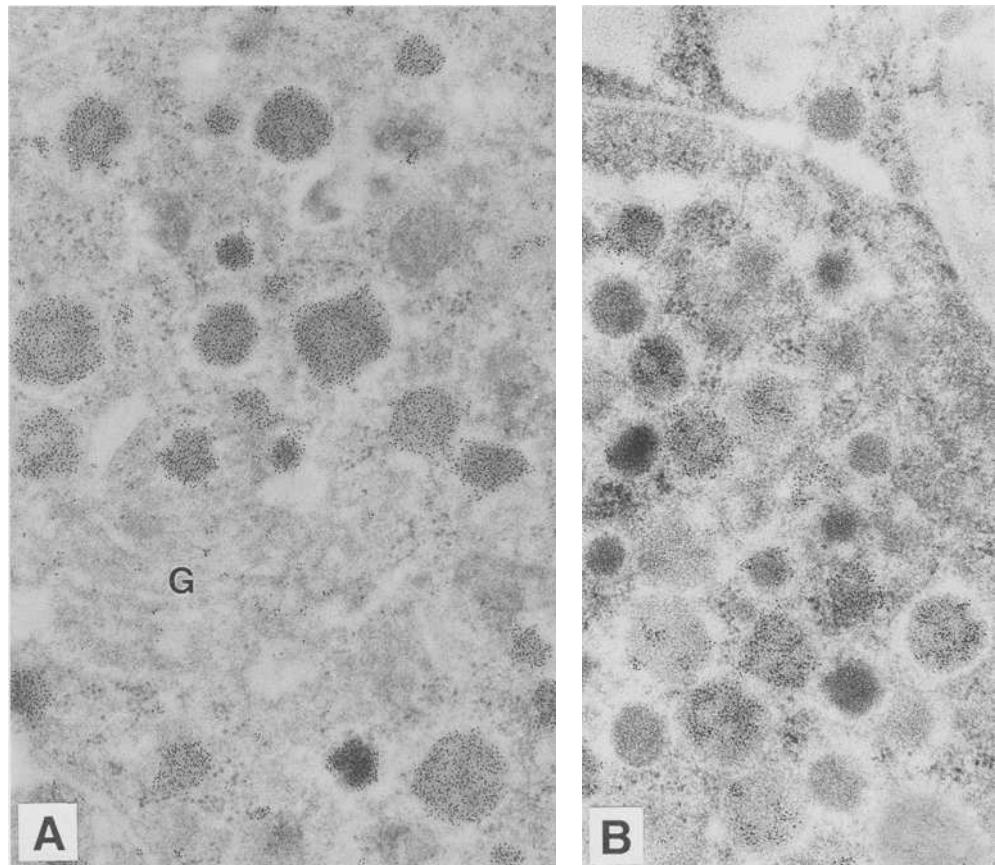


Fig. 3. Immunoelectron microscopic demonstration of proinsulin and insulin in a human insulinoma, immunogold labeling, Lowicryl K4M thin sections. Insulin immunoreactivity as indicated by the electron dense gold particles is present over the Golgi apparatus (G) and electron dense core secretory granules (A). Immunogold labeling for proinsulin can be found also in secretory granules in the periphery of the insulinoma cells (B).

biological behavior or the potential for metastatic disease. However, most of the proposed markers have been shown to be only of limited value in the daily practice [41] and the individual prognosis of a given patient with an EPT cannot be satisfactorily predicted by conventional histological parameters or immunohistochemical expression patterns.

Thus, in a study on 53 EPTs (38 benign, 15 malignant) we could demonstrate that the expression of polysialylated NCAM, NCAM protein isoforms, and beta-1,6-branched, asparagine-linked oligosaccharides, which have been associated with increased aggressiveness, metastatic potential, and survival in other tumor types [42], are not suitable to distinguish between malignant and benign EPTs. Polysialylated NCAM was not detectable in benign and malignant insulinomas, and the NCAM protein isoform pattern was similar as well. Furthermore, 11/15 (74%) of the malignant and 23/38 (63%) of the benign tumors exhibited staining for beta-1,6-branches [43]. In another study on 134 neuroendocrine tumors of different locations, including 27 EPTs (6 insulinomas, 10 gastrinomas, 1 glucagonoma, 3 somatostatinomas, 7 VIPomas), we could demonstrate that not only gastrinomas and nonfunctioning EPTs (as reported by other groups) but also other types of EPTs may express the glycoprotein CD44 and its isoforms and that the expression of CD44 is not associated with a malignant phenotype or tumor progression as reported for other types of human tumors [44].

Clonal Composition of EPTs

In a study on the clonality of 34 malignant and benign EPTs of different types from female patients [44], we have shown that 7/20 of the informative tumors exhibited a monoclonal (35%), 10/20 a

polyclonal, 1/20 an oligoclonal pattern, and 2/20 a loss of heterozygosity (LOH) at the Xq12-locus. When comparing benign and malignant neoplasms, benign tumors more often exhibited a polyclonal (5/7) pattern, while malignant tumors more frequently showed a monoclonal (8/13), an oligoclonal (1/20) pattern, or LOH (2/20). These results are consistent with the hypothesis that EPTs might primarily be polyclonal or oligoclonal neoplasms, which are eventually outgrown by a more aggressive cell clone that gives rise to invasive growth and/or metastasis. Furthermore, we postulated that the X-chromosome, which was lost in two of the examined highly malignant EPTs, might play a role in the progression of EPTs by inactivation of a not yet identified tumor suppressor gene [45].

Molecular Pathogenesis of EPTs

Although the molecular basis of the familial EPTs (which are associated with MEN1 and VHL) has recently been established [46,47], only little is known about the oncogenesis and the molecular basis of progression of sporadically occurring EPTs. A small number of published studies indicate that in contrast to other human tumors, the activation of oncogenes is not an early event in EPTs. It was shown that, for example, the protein- and RNA-expression (but not mutations) of *H-ras* and *K-ras* is associated with the progression of sporadic EPTs and oncogenes (such as *fos*, *c-myc*, *M-myc*, and *sis*) or tumor suppressor genes (such as *p53* and *Rb*), which are frequently mutated or activated in other human tumors, appear not to be involved in the neoplastic transformation of EPTs, or merely involved as a late event [48–51]. Furthermore, in an analysis of 112 sporadic neuroendocrine tumors for *RET*–

protooncogene mutations, we found that the 17 examined EPTs lacked mutations in all five examined *RET*-protooncogene exons, which indicates that this gene is not generally important in the tumorigenesis of EPTs [52].

Molecular and cytogenetic analysis have led to the identification of a number of chromosomal alterations in EPTs. However, no consistent pattern has been noted so far. There are indications that certain chromosomal abnormalities are more frequently encountered in EPTs.

Several LOH studies have shown that the chromosome 11q13 region is deleted in a subset of EPTs [51,53]. The chromosomal region 11q13 is the locus of the recently identified *MEN1* gene. Thus, it is tempting to assume that *MEN1* mutations are also involved in the formation of sporadic EPTs. However, we and others [53–55] could only identify a small percentage of sporadic EPTs harboring somatic mutations in the *MEN1* gene, indicating that this gene is only involved in a subset of EPTs and that the majority of EPTs are probably caused by other genetic lesions. In the study of Zhuang et al. on 12 sporadic insulinomas, only two tumors exhibited an inactivating *MEN1* gene mutation [53] and in our own series of 30 EPTs we have observed a mutation rate of only 4/30 (13.3%). The series also included nine insulinomas, which exhibited frequencies of 44.4% for LOH (4/9) and 11.1% for mutations (1/9) [54–56].

LOH studies on other loci did not reveal any other concise candidate gene for the initiation or progression of sporadic EPTs [50,51,57]. For example, it recently has been demonstrated that loss of chromosome 3p25 in sporadic EPTs is associated with a more aggressive phenotype [58] and that no mutations of the *VHL* gene (which is located in this particular region of the

chromosome) is present in these tumors [58]. Further chromosomal loci that have been occasionally found deleted in sporadic EPTs include chromosome 1p, 5q, 9q, 7q, and 16q [60,61].

Only a few publications exist on cytogenetic aberrations in EPTs [62,63]. They were mainly observed in cultured tumor cells and the identified changes were not confirmed by LOH or FISH methods. One study on cultured EPTs of nine patients showed a multitude of chromosomal aberrations in half of the investigated tumors. However, no consistent aberration has been noted and cytogenetical findings were not correlated with clinical data [63]. Another recently published study on 16 insulinomas from transgenic mice (with a SV40-large-T-antigen transgene under the control of the rat insulin promoter) reported LOH on the mouse chromosomes 9 and 16, which are homologous to the human chromosome regions 6p, 3p, 3q and 22q, 21q, 3q, respectively, in a majority of examined tumors [61]. In the same study, additional losses on chromosomes 2, 6, 7, 11, 13, and 14 could be identified using comparative genomic hybridization (CGH). In our own CGH study, we have analyzed 44 benign and malignant EPTs, in order to correlate the overall number of genetic alterations with clinical and histopathological parameters and to identify chromosomal regions that might harbor genes involved in EPT initiation and progression. Aberrations were found in 36 EPTs and chromosomal losses were slightly more frequent than gains. The most frequent losses involved Y, 6q, 11q, 3p, 3q, 11p, 6p, 10q, and Xq, and most common gains included 7q, 17q, 5q, 14q, 7p, 9q, 17p, 20q, 12q, and Xp. A correlation was found between the total number of genetic changes per tumor and both tumor size and disease stage. In particular, losses of 3p and

6q and gains of 14q and Xq were found to be associated with metastatic disease. Furthermore, characteristic patterns of genetic changes were found in the various EPT subtypes, e.g., 6q loss in malignant insulinomas, indicating that these groups might evolve along genetically different pathways. The highlighted genetic aberrations, including the newly found involvement of 6q losses and sex chromosome alterations, should stimulate the further analysis of these chromosomal regions, which may lead to the discovery of novel genes important in the tumorigenesis and evolution of EPTs [64,65].

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